Lipid and Genetic Predictors of Midlife Cognitive Decline in Treated HIV-1 Infection

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Abstract

Cognitive impairment affects 20-50% of HIV-1-infected (HIV+) adults on antiretroviral therapy (ART), and the search for protective factors has intensified as the population ages [1, 2]. Predictors of cognitive impairment in HIV+ populations are unknown, and multiple factors including ongoing low levels of viral replication, systemic inflammation, coinfection with Hepatitis C, genetic predisposition, irreversible CNS injury prior to ART, and neurotoxicity of ART regimens are potential candidates for influencing cognitive outcomes [3-5]. Furthermore, aging modifies inflammatory responses through molecular mechanisms that include altered lipid metabolism, mitochondrial function, and oxidative stress, coined "inflammaging" [6, 7]. The factors associated with HIV-associated neurocognitive impairment has been largely sought in cohort studies with cross-sectional designs, but these studies are limited by demographic differences that can influence neuropsychological test results, and difficulty in making associations between outcomes and exposures of long duration [4, 8-11]. To date, longitudinal studies investigating similar or overlapping age- and HIV-related mechanisms contributing to cognitive decline in older ART-suppressed HIV+ adults are lacking.

In the first portion of this thesis, we describe studies using a mixed-effects approach to model age-related cognitive trajectories of ART-adherent HIV+ and HIV- adults modified by time-varying lipid indices and APOE ε4 genotype. This was a prospective study of HIV+ and HIV- men enrolled in the Multicenter AIDS Cohort Study from 1996-2010, and we showed that dyslipidemia and APOE ε4 allele are independent risk factors for cognitive decline in ART-adherent HIV+ men between ages 50-65. The findings are significant because they identify novel risk factors for midlife cognitive decline in HIV+ men on suppressive ART and suggest that clinical management of dyslipidemia may be an effective strategy to reduce cognitive decline in HIV+ men over age 50. Systemic inflammation, cardiovascular disease (CVD) and APOE ε4 genotype are associated with age-related cognitive decline in the general population [12-16], but the combined extent to which they account for midlife cognitive decline in HIV-infection remains unknown. In the second portion of this thesis, we discuss our strategy to quantify the extent to which cognitive decline is explained by inflammation, CVD, and APOE ε4 genotype in older ART-adherent HIV- adults, and determine how these variables influence the transition point from normal aging to cognitive decline in older ART-suppressed HIV+ individuals.

CHAPTER Ia:

Lipid Profiles and APO ε4 Allele Impact Midlife Cognitive Decline in ART-Adherent HIV+ Men

(This work has been submitted for publication)

Lipid Profiles and APOE4 Allele Impact Midlife Cognitive Decline in HIV-Infected Men on Antiretroviral Therapy

Shibani S. Mukerji[1,2]; Joseph J. Locascio[2]; Vikas Misra[1]; David R. Lorenz[1]; Alex Holman[1]; Anupriya Dutta[1]; Sudhir Penugonda[3]; Steven M. Wolinsky[3]; Dana Gabuzda[1*]

[1]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA, US; [2]Department of Neurology, Massachusetts General Hospital, Boston, MA, US; [3] Division of Infectious Diseases, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, US

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Introduction

The population of HIV-1-infected (HIV+) individuals over age 50 is growing due to effective antiretroviral therapies (ART), and focus has shifted to prevention and management of age-related comorbidities. Dyslipidemia is common among people living with HIV infection (PLWH) in the current ART era. Persistent elevations in triglycerides and total cholesterol, and reductions in high-density lipoprotein (HDL) levels are detected in HIV+ cohorts, while elevations in low-density lipoprotein (LDL) are less consistent [17, 18]. Previous studies suggest that elevated total cholesterol or low HDL levels are associated with increased risk of late onset dementia in the general population [19, 20]. Furthermore, high total cholesterol was implicated as a risk factor for lower cognitive scores in PLWH and worsening HIV-1 associated neurocognitive disorders (HAND) [21], but the longitudinal effects of lipid levels on cognitive decline in ART-treated older HIV+ individuals are unknown.

The main cholesterol transporter in the central nervous system is Apolipoprotein E (APOE), a structural component of very low density lipoproteins and HDL involved in catabolism of triglyceride-rich lipoproteins [22]. Three major APOE isoforms are encoded by the ε2, ε3, and ε4 alleles, with worldwide frequencies of ~8%, 78%, and 14%, respectively [23]. The ε4 allele is the most important genetic risk factor for Alzheimer's disease (AD), and is a risk factor for age-related cognitive decline in the general population [12]. The relationship between APOE genotype and HAND is unclear due to conflicting results [3, 4, 9-11, 24-32]. While some cross-sectional studies suggest that the ε4 allele increases risk for HAND over age 50 [27, 31], others found no significant cognitive effect of the ε4 allele in HIV+ adults [3, 24, 26, 28, 32]. The ε4 allele has been associated with hypercholesterolemia, but no studies have examined whether ε4 genotype interacts with cholesterol levels to influence cognitive decline in aging PLWH.

It is critical to understand when lipids and APOE ε4 status modify cognitive performance among ART-treated HIV+ adults, since these factors can guide clinical practice and trial design. Here, we performed longitudinal growth modeling on a cohort of ART-adherent HIV+ and HIV- men between the ages 50-65 years using a mixed-effects model approach to estimate the impact of lipid profiles and APOE ε4 allele on midlife cognitive trajectories. We then examined whether cholesterol levels and ε4 genotype interact to influence risk of midlife cognitive decline.

Methods

Data Source

This was a prospective study using data from the Multicenter AIDS Cohort Study (MACS), an observational cohort of HIV+ and HIV- men who have sex with men. Interviews, physical examinations, and biological specimens were collected in biannual visits; neuropsychological (NP) examinations began in 1986. Details of the study design and enrollment patterns have been previously described ([33], Supplementary Methods). The Institutional Review Boards at each of the clinical sites approved the research, and subjects signed a written statement of informed consent. The MACS public data is released annually (http://www.ntis.gov/search/index.aspx) [33]; the p23 release was translated to a local SQL database and used in these analyses.

Study Population

This study was restricted to MACS visits between January 1996 and December 2010. A sequential process was performed to define the study cohort of 789 men between ages 50-65 (Figure 1; Supplementary Data). Among 3346 men with visits from 1996-2010, 1250 were outside the age for eligibility, had a history of CNS opportunistic infections, or reported cocaine, crack, or heroin use at >50% of visits during the study period while 653 were excluded due to ART adherence < 95% in follow-up, missing data, and other exclusion criteria (Fig. 1; Supplementary Methods). For inclusion, HIV+ participants had to be on ART for at least 1 year prior to baseline visit and have plasma viral load (VL) < 400 copies/ml at baseline visit. HIV- controls were matched to HIV+ cases with the MatchIt package in R (Version 2.4-21; http://gking.harvard.edu/matchit) [34]. Matched covariates included age at study entry, black race, education level, alcohol use, and smoking.

Measures of cognitive function

A battery of 15 NP tests measuring cognitive domains related to HAND was used to generate a composite cognitive summary score [35]. Individual tests were converted to z-scores using the test's mean and standard deviation (SD) from HIV- and HCV-antibody negative men ages 45-49 years old stratified by education level as reference norms. The summary cognitive score created to capture heterogeneity in

performance included: 1) executive function (trail-making part B, Stroop interference); 2) perceptual speed (Symbol Digit Modalities Test, Stroop color naming and word naming, trail-making part A); 3) attention and working memory (CalCAP reaction time measures); 4) verbal learning and memory (Rey Auditory Verbal Learning test (RAVLT) sum of trials 1-5; RAVLT immediate recall; RAVLT delayed recall); 5) motor (Grooved Pegboard, both scores) (Supplementary Table 1). The following covariates with potential for confounding given published associations with HIV infection or cognitive function were used in adjusted models: baseline age (years), Shipley WAIS IQ-Equivalent score (IQ), Centers for Epidemiologic Studies Depression (CES-D) score, smoking, and CD4 cell count.

Genotyping

Genomic DNA extraction and genotyping of *APOE* single nucleotide polymorphisms (SNPs) rs429358 [C/T] and rs7412 [C/T] from individuals within the MACS has been described [32]. Genotyping was conducted using TaqMan OpenArray technology. Arrays were imaged after amplification on OpenArray NT images, genotypes ascribed after clustering VIC and FAM signals (STATA 12.1, College Station, TX) and used to determine APOE alleles. APOE genotype was available for 350 participants.

Statistical Methods

Cohort characteristics were described using means and SDs or median and interquartile range (IQR) depending on the distribution of variables. Simple univariate/bivariate tests were conducted using *t* tests, Wilcoxon rank sum tests, ANOVA, Pearson χ^2 or Fisher exact tests. The association between total cholesterol, HIV infection, and change in cognitive score was examined using mixed-effects models with interaction terms for total cholesterol with time, HIV infection with time, and their joint interaction with time; total cholesterol was analyzed as a time-varying covariate. A quadratic model was used to allow for potential acceleration in the rate of decline. Continuous variables included baseline age at study entry, CES-D score, and IQ score; CD4 cell count was examined as a time-varying covariate. Smoking and HIV infection were analyzed as binary covariates. A backwards elimination procedure was used to identify significant longitudinal relationships among predictors ($P < 0.05$ cut-off). The effect of APOE ε4 allele was explored in an independent mixed-effects model, with adjustment for the same covariates. APOE ε4 status was modeled as a categorical covariate (ε4 carrier, ε4 noncarrier, and unknown/ε2 homozygotes).

The decision to categorize the ε2 allele separately was made prior to analysis given its protective cognitive effects which may falsely underestimate cognitive decline in ε4 noncarriers [22]. Random predictors were correlated intercepts and linear slopes of time. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

Clinical Characteristics

Clinical characteristics of the study cohort are shown in Table 1 (N= 789 men; 273 HIV+, 516 HIV-). The median age was 51 years at study entry (IQR: 50-55 years) with a mean follow-up of 6.3 years. Eighty-one percent self-identified as non-Hispanic white, and 14% as black. HIV+ and HIV- men had similar proportions with >12 years of education (*P*=0.35). HIV+ men had higher mean baseline CES-D scores (9.7 *vs*. 8.5) and greater proportion with scores ≥16 (22% *vs*. 18%; P=0.08), a cut-off for high depressive symptoms. The median CD4 count was 514 at baseline and median nadir CD4 count was 387 cells/ml (IQR: 265-536) in follow-up; 70% maintained viral suppression (<50 copies/ml with ≤2 blips, blip ≥400 copies/ml; Supplementary Figure 1). Baseline total cholesterol and LDL levels were similar between groups, while HIV+ men had higher triglycerides and lower HDL than controls (*P*<0.001). Among HIV+ men, 51% were on a statin for at least one year.

Lipids and Cognitive Decline

Time-related terms reflecting the association between total cholesterol levels and cognitive decline between ages 50-65 years are summarized in Table 2. While the estimated rate of cognitive decline accelerated with increasing cholesterol levels in both groups, HIV+ men had a faster rate of decline compared to HIV- controls (*P*=0.003; Figure 2). Figure 2 depicts the estimated annual rate of decline for a 50 year old man with and without HIV infection, illustrating two main findings: 1) On average, HIV+ men with higher total cholesterol levels have faster rates of cognitive decline than HIV+ men with lower levels; 2) The rate of cognitive decline in HIV+ men ages 50-65 years is differentially modified by cholesterol compared to HIV- men of the same age, IQ, baseline CES-D score, smoking status, and CD4 count. Given that higher cholesterol levels in HIV+ men were marginally associated with better cognitive scores at the intercept (*P*=0.05; Supplementary Table 2), deleterious effects of elevated cholesterol most

likely occurred after age 50. Older age and baseline CES-D scores correlated with lower cognitive scores; IQ was associated with higher scores (Supplementary Table 2).

In *post-hoc* analyses, total cholesterol was replaced with time-varying LDL, HDL, or $\log_{10}$ triglycerides, and associations with cognitive scores examined. Higher LDL and triglycerides were associated with a steeper slope of cognitive decline, while elevated HDL levels attenuated the rate of cognitive decline in HIV+ men (Figure 2 and Table 2). The association between total cholesterol and decline in specific cognitive domains was examined in secondary analyses for composite scores of executive function, perceptual speed, verbal memory, attention and working memory, and motor speed. Higher total cholesterol was associated with a steeper slope of decline in attention and working memory ($P < 0.001$), and marginal significance for verbal memory ($P=0.05$; Supplementary Table 2). Additionally, the association between cholesterol level and the rate of decline among HIV+ subjects remained significant after the exclusion of two subjects with baseline cognitive z-score <-2 (data not shown).

APOE ε4 Allele and Cognitive Decline

Cohort characteristics of APOE ε4 carriers and noncarriers were similar to the larger study cohort (Supplementary Table 3), and ε4 genotype frequencies were comparable between groups (Figure 3A). Among HIV+ ε4 carriers vs. noncarriers, there were no differences in median baseline CD4 count, CD4 nadir during follow-up, or ART medications used, but HIV+ ε4 carriers had higher baseline triglyceride levels ($P<0.001$). While longitudinal decline in cognitive scores was observed among all HIV+ individuals, the rate of decline accelerated among HIV+ APOE ε4 carriers ($P=0.01$; Table 3). Divergent estimated slopes in Figure 3B illustrate that the estimated cognitive trajectory for HIV+ ε4 carriers deviates rapidly from HIV+ ε4 noncarriers and HIV- controls between ages 50-65. Given that there were no significant differences in the intercept between HIV+ carriers and noncarriers at study entry (Supplementary Table 4), cognitive decline for HIV+ ε4 carriers is expected to start after age 50. In *post-hoc* analyses accelerated rate of decline in perceptual speed, but no other cognitive domains, was estimated for HIV+ ε4 carriers ($P= 0.03$; Supplementary Table 4).

Given that cholesterol levels and APOE ε4 genotype was associated with cognitive decline in HIV+ men, we next examined whether these covariates interact to influence the rate of decline. The 3-way interaction

term between HIV infection, cholesterol, and time (P=0.002; Table 3) remained significant for cognitive decline among HIV+ APOE ε4 carriers. While accelerated rates of decline were estimated among HIV+ APOE ε4 carriers vs. noncarriers (P<0.01), the annual rate of decline among ε4 carriers was not further modified by cholesterol levels (P=0.9; Figures 3C and D, Table 3, and Supplementary Table 5). Thus, cholesterol levels and presence of the ε4 allele have independent effects on cognitive decline in HIV+ subjects, and do not substantially influence their respective associations.

Discussion

In this prospective study, elevated cholesterol, LDL, and triglyceride levels were associated with faster rates of cognitive decline in ART-adherent HIV+ men ages 50-65, while higher HDL attenuated cognitive decline. The APOE ε4 genotype was associated with accelerated cognitive decline in HIV+ ε4 carriers over age 50, approximately a decade earlier than reported for HIV- ε4 carriers [23], suggesting that the interaction between treated HIV-infection and the ε4 genotype is a significant risk factor for earlier onset of cognitive decline. Cholesterol levels and the APOE ε4 genotype had independent effects on the rate of decline among treated HIV+ but not HIV- men, and are therefore unlikely to be redundant risk factors. In aggregate, these findings suggests that control of dyslipidemia may reduce the risk of midlife cognitive decline in aging PLWH on ART, and the APOE ε4 genotype likely influences cognitive trajectories via mechanisms distinct from its effects on lipid metabolism.

HIV+ individuals are at increased risk for dyslipidemia due to HIV infection and ART, and have higher rates of cardiovascular disease and metabolic syndrome [8, 36]. Consistent with previous studies, triglyceride levels were higher, while HDL levels were lower in HIV+ vs. HIV- subjects in the study cohort. We tested the relationship between time-varying cholesterol levels and cognitive decline, and showed that for every 10 mg/dl increase in total cholesterol or LDL between ages 50-65, the rate of cognitive decline among HIV+ men increased. We also demonstrated a positive relationship between time-varying HDL levels and longitudinal cognitive performance in HIV+ subjects. While published reports on the relationship between lipids and cognitive decline in the general population are mixed [37], the association between HDL and higher cognitive scores in midlife HIV+ men is similar to findings in older HIV-uninfected cohorts [38, 39]. HDL-like lipoproteins are found in CSF, are lower in those with AD or APOE ε4, and may be protective against cognitive decline [37]. HDL is proposed to play a role in mitigating

oxidative stress, metabolizing oxidized lipids, and reducing LDL-induced inflammation [40]. Together with findings from preceding studies demonstrating alterations in CSF lipid metabolism among HIV+ adults [41, 42], these analyses highlight the importance of future investigation to identify mechanisms by which altered lipids affect cognitive aging and potential strategies for therapeutic intervention.

While our findings suggest that the APOE ε4 allele has a substantial effect on cognitive decline in older men with treated HIV infection, accelerating a downward trajectory after age 50, they differ from those of two previous longitudinal studies [26, 32]. Burt *et. al.* did not identify an association between the APOE ε4 allele and HIV-associated dementia in subjects on early ART regimens and Becker *et. al.* recently reported no association between the Ɛ4 allele and time to impairment or death. However, there are key methodological differences between study designs that should be taken into account when comparing the aforementioned results to the present study. We studied ART-adherent HIV+ men over age 50, included HIV-controls well-matched for demographics and lifestyle factors, and allowed for acceleration in the rate of decline. Our model predicts that while all groups are estimated to decline over time, there is a complex, nonlinear relationship between time and cognitive performance among older HIV+ ε4 carriers. Statistical models in prior longitudinal studies relied on assumptions that the relationship between predictors, time, and risk for cognitive impairment remains constant. As such, time or age-dependent effects of the ε4 allele may have been underestimated in later follow-up.

The lack of biomarkers specific for HAND or cognitive aging has resulted in controversy regarding accurate identification of at-risk HIV+ individuals with undetectable plasma HIV-1 RNA levels. Our findings suggest that dyslipidemia and ε4 allele are risk factors for midlife cognitive decline in ART-treated HIV+ adults over age 50. In addition to its role in Aβ homeostasis, APOE modulates neuroinflammation and oxidative injury in an isoform-specific manner [43, 44]; these effects may be augmented in aging PLWH, especially given that HIV-related metabolic syndrome and abdominal obesity are associated with CSF immune activation markers and cognitive impairment [8, 45]. Superimposed cognitive aging effects related to dyslipidemia or ε4 genetic susceptibility, HIV-related neuroinflammation, and oxidative injury may increase vulnerability to midlife cognitive decline among ART-suppressed HIV+ individuals. Total cholesterol levels did not further moderate decline in HIV+

APOE ε4 carriers, suggesting that cholesterol and ε4 allele have independent effects on cognitive decline via mechanisms that may involve cerebrovascular disease, in addition to other mechanisms.

This study has several limitations, including those inherent to longitudinal observational studies such as selection, survivorship, and severity bias reflected in characteristics of the MACS study population. These findings require replication in study populations with other demographic characteristics. The study was limited to men, predominantly with > 12 years education. Epidemiological studies report greater risk of clinical conversion from healthy aging to mild cognitive impairment or AD in female APOE ε4 carriers compared to males [46], highlighting the need for similar analyses in HIV+ women. Low education level is a known predictor for decline to symptomatic HAND [35] and higher educational attainment may provide some protection against effects of the ε4 allele by increasing cognitive reserve. Nonetheless, despite high education levels, HIV+ men remained vulnerable to faster rates of decline compared to HIV- controls in the presence of high cholesterol or the ε4 allele.

Our findings suggest that clinical management of dyslipidemia in ART-adherent HIV+ individuals may reduce the risk of midlife cognitive decline, and the window of opportunity likely occurs at a younger age than in the general population. A current consideration for adjuvant treatment is statins. Statins block conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor for cholesterol, and have pleiotropic effects including reducing inflammatory responses and improving endothelial function. Statins have not reduced AD risk in several randomized clinical trials [47], but these results should be interpreted with caution when considering management of dyslipidemia in HIV infection. Statins primarily target LDL, while cognitive impairment shows stronger associations with HDL [38, 39]. Altered HDL functionality is associated with immune activation in HIV infection [48], and findings presented here estimate that higher HDL levels attenuate cognitive decline. Given impressive efforts that have improved survival among HIV+ individuals, this study underscores the importance of lipid profiles and APOE ε4 allele to midlife cognitive health in aging HIV+ adults and suggest that clinical management of dyslipidemia may be an effective adjunctive strategy to reduce cognitive decline in ART-treated HIV+ individuals.

Table 1: Cohort Characteristics

	All Subjects	HIV-	HIV+	*P* value
No. of patients	789	516	273	NA
Length of Follow-up, mean (SD), years	6.3 (3.2)	6.0 (3.2)	6.8 (3.1)	<0.01
Demographics				
Baseline Age, median (IQR) years	51.0 (50-55)	51.5 (50-55)	50.0 (50-52)	<0.001
Race, No. (%)				
White	636 (80.6)	429 (83.1)	207 (75.8)	<0.01
Black	112 (14.2)	62 (12.0)	50 (18.3)	
Hispanic or Latino	21 (2.7)	15 (2.9)	6 (2.2)	
Other	15 (1.9)	10 (1.9)	5 (1.8)	
Education >12 years, No. (%)	702 (89.0)	463 (89.7)	239 (87.5)	0.35
Shipley WAIS IQ- Equivalent, mean (SD)	109.5 (8.9)	109.1 (10.7)	106.5 (11.3)	<0.01
Depression Profile				
Baseline CES-D Score, mean (SD)	8.9 (9.6)	8.5 (9.5)	9.7 (9.8)	0.13
Baseline CES-D Score ≥ 16, No. (%)	151 (19.1)	92 (17.8)	59 (21.6)	0.08
Substance Use, No. (%)				
Smoking (Highest use on ≥ 2 visits)				0.26
None	574 (72.8)	386 (74.8)	188 (68.9)	
<1/2 pack per day	66 (8.4)	38 (7.4)	28 (10.3)	
1/2 - 2 packs per day	146 (18.5)	90 (17.4)	56 (20.5)	
Alcohol (Highest use on ≥ 2 visits)[1]				0.07
None-Light	111 (14.1)	71 (13.8)	40 (14.7)	
Occasional-Moderate	566 (71.7)	379 (73.4)	187 (68.5)	
Heavy/Binge	100 (12.7)	55 (10.7)	45 (16.5)	
Baseline Lipid Profile, Mean (SD), mg/dl				
Total Cholesterol	197.1 (40.4)	195.1 (36.3)	201.1 (47.4)	0.11
LDL	115.4 (34.8)	117.2 (33.7)	111.9 (36.8)	0.08

HDL	47.6 (13.3)	49.0 (12.4)	44.8 (14.4)	<0.001
Triglycerides	161.0 (123.1)	136.3 (95.5)	212.7 (154.8)	<0.001
Lipid-lowering Medication, No. (%)[3]				
Statin	304 (38.5)	164 (31.8)	140 (51.3)	<0.001
Fibrate	78 (9.9)	23 (4.5)	55 (20.1)	<0.01
Niacin	38 (4.8)	21 (4.1)	17 (6.2)	0.24
HCV Antibody Positive, No. (%)	74 (9.4)	35 (6.8)	39 (14.3)	<0.01
HIV Disease Characteristics, Median (IQR)				
Baseline CD4 (cells/μl)[2]	805 (569-1055)	951 (743-1174)	514 (333-684)	<0.001
CD4 nadir (cells/μl) in study	623 (431-840)	726 (580-916)	387 (265-536)	<0.001
Baseline HIV-$_1$ RNA VL (copies/ml)[2]			40 (40-40)	
Baseline CPE Score[2]			7.0 (6-9)	
Antiretroviral Medication, No. (%)[3]				
Azidothymidine			95 (34.8)	
Efavirenz			114 (41.8)	
Protease Inhibitor			190 (69.6)	
ddI, d4T, ddC			106 (38.8)	
Abacavir			96 (36.2)	

P values <0.05 were considered significant.

[1] Alcohol Use: Light= <1 drink/week; Occasional-Moderate= 1-14 drink(s)/week; Heavy= >14/week, Binge= ≥5 drinks/one sitting/month

[2] Baseline values: first visit or within 6 months of study period

[3] Self-reported Lipid-lowering medication and ART medication used on ≥ 2 visits

Abbreviations: SD= standard deviation; IQR: Quartile1 – Quartile3; NA= not applicable; CES-D= Center for Epidemiological Studies Depression Scale; LDL= low-density lipoprotein; HDL=high-density lipoprotein; HCV= hepatitis C virus; HIV= human immunodeficiency virus; VL= viral load; CPE= CNS Penetration Effectiveness[49]; ddI= Didanosine; d4T= Stavudine; ddC=Zalcitabine

Table 2: Associated Effect of Lipids on the Annual Rate of Cognitive Decline

Total Cholesterol	**Estimate**	**SE**	***P* value**
HIV+ * Years in Study	0.0613	0.0226	0.007
Total Cholesterol (10 mg/dl) * Years in Study	0.0040	0.0016	0.112
Total Cholesterol (10 mg/dl) * Years in Study * Years in Study	-0.0003	0.0001	0.043
Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**-0.0034**	**0.0011**	**0.003**
LDL			
HIV+ * Years in Study	0.0423	0.0170	0.013
LDL (10 mg/dl) * Years in Study	0.0022	0.0018	0.995
LDL (10 mg/dl) * Years in Study * Years in Study	-0.0002	0.0002	0.371
LDL (10 mg/dl) * HIV+ * Years in Study	**-0.0043**	**0.0014**	**0.002**
HDL			
HIV+ * Years in Study	-0.0460	0.0202	0.024
HDL (10 mg/dl) * Years in Study	-0.0006	0.0053	0.390
HDL (10 mg/dl) * Years in Study * Years in Study	0.0001	0.0005	0.819
HDL (10 mg/dl) * HIV+ * Years in Study	**0.0098**	**0.0043**	**0.022**
Triglycerides (Log_{10} mg/dl)			
HIV+ * Years in Study	0.0949	0.0450	0.036
Triglycerides * Years in Study	0.0599	0.0259	0.121
Triglycerides * Years in Study * Years in Study	-0.0047	0.0023	0.041
Triglycerides * HIV+ * Years in Study	**-0.0424**	**0.0205**	**0.039**

Adjusted for age, Shipley WAIS IQ- Equivalent Score, and CES-D at study entry, smoking status, and CD4 count. Lipid estimates except triglyceride levels were interpreted in 10 mg/dl increments. A negative estimate indicates a steeper slope of cognitive decline.

R2 for fixed effects= 0.25, P<0.001; R2 including random terms=0.94, P<0.0001

The squared Pearson correlations of predicted values from fixed or fixed and random terms versus actual values of the dependent variable indicate the percent of variance accounted for by predictors (reported for total cholesterol).

Abbreviations: LDL= low density lipoprotein, HDL= high density lipoprotein, SE= standard error,
* indicates an interaction

Table 3: Effect of APOE ε4, Total Cholesterol, and HIV Infection on the Rate of Cognitive Decline

Model 1 (N=542):	**Estimate**	**SE**	***P* value**
HIV+ * Years in Study	-0.0104	0.0168	0.266
HIV+ * Years in Study * Years in Study	0.0002	0.0016	0.003
APOE ε4 Carrier * Years in Study	0.0227	0.0243	0.022
APOE ε4 carrier * Years in Study * Years in Study	-0.0008	0.0022	0.002
HIV+ * APOE ε4 carrier * Years in Study	0.0519	0.0355	0.300
HIV+ *APOE ε4 carrier * Years in Study * Years in Study	**-0.0106**	**0.0035**	**0.010**
Model 2 (N=245):			
APOE ε4 Carrier * Years in Study	-0.02388	0.06127	0.8136
HIV+ * Years in Study	0.1452	0.04568	0.0005
Total Cholesterol(10 mg/dl) * Years in Study	0.00214	0.00125	0.8417
Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**-0.0056**	**0.00184**	**0.0021**
APOE ε4 Carrier * HIV+ * Years in Study	0.0266	0.07136	0.71
APOE4 carrier * HIV+* Years in Study * Years in Study	**-0.01099**	**0.003421**	**0.0058**
APOE ε4 Carrier * Total Cholesterol (10 mg/dl) * Years in Study	0.00192	0.00252	0.3598
APOE ε4 Carrier * HIV+ * Total Cholesterol (10 mg/dl) *Years in Study	-0.00004	0.000308	0.897

Adjusted for age at study entry, Shipley WAIS IQ- Equivalent Score, CES-D at study entry, smoking status, and CD4 count. Total cholesterol was interpreted in 10 mg/dl increments. APOE ε4 was modeled as a categorical variable (ε4 carrier, ε4 noncarrier or unknown/ε2 homozygous) in Model 1. Model 2 included subjects with known APOE ε4 genotype.

Model 1: R2 for fixed effects= 0.26, P<0.001; R2 including random terms=0.97, P<0.0001

Model 2: R2 for fixed effects= 0.28, P<0.001; R2 including random terms=0.93, P<0.0001

The squared Pearson correlations of predicted values from fixed or fixed and random terms versus actual values of the dependent variable indicate the percent of variance accounted for by predictors.

Abbreviations: APOE= Apolipoprotein E, CES-D= Center for Epidemiological Studies Depression Scale, IQ =Shipley WAIS IQ- Equivalent Score, SE= standard error, * indicates an interaction

Figures

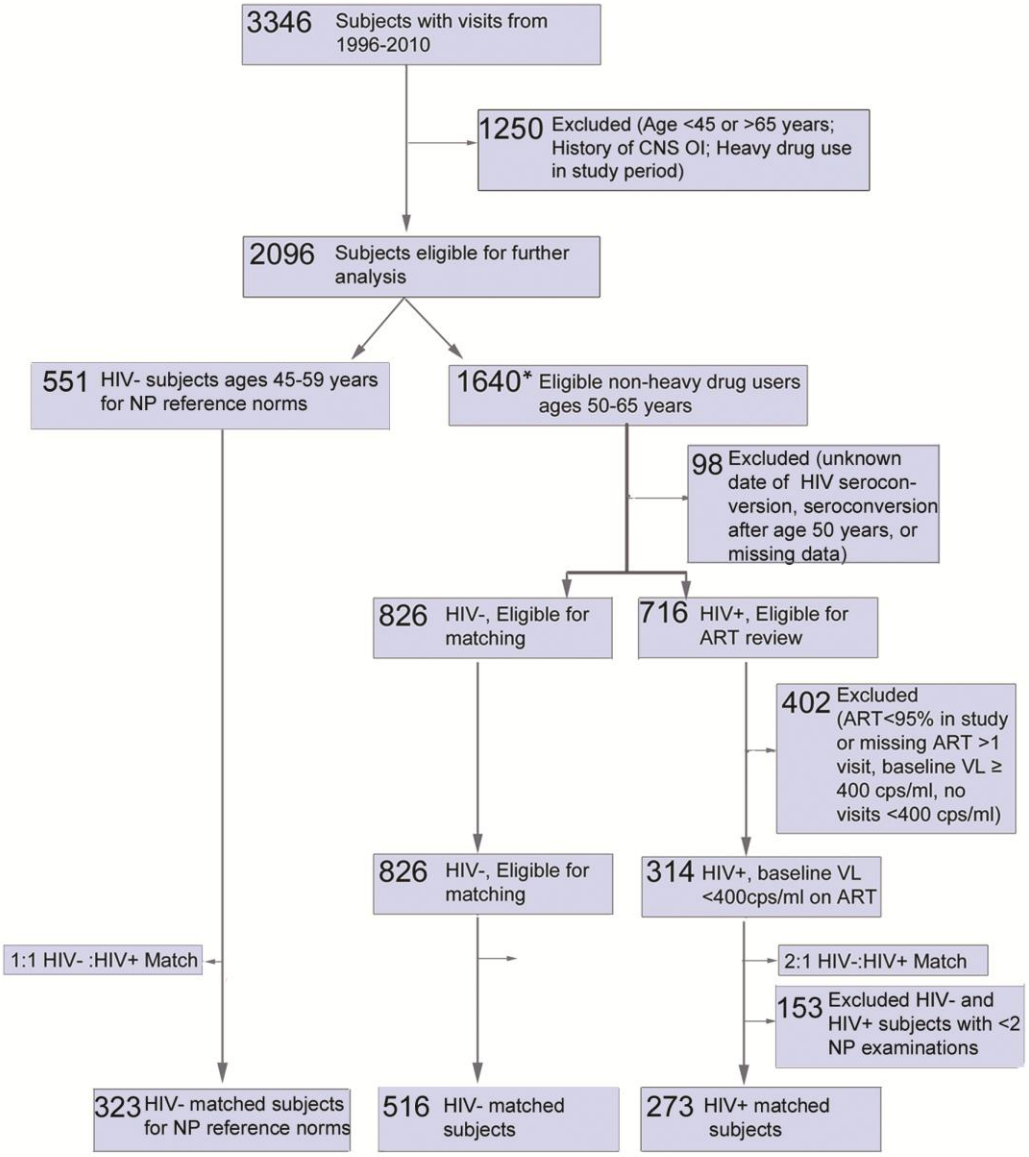


Figure 1: Selection of the HIV- and HIV+ Study Cohort

Subject enrollment and sequential application of inclusion and exclusion criteria to define the study population. *Subjects with neuropsychological scores between ages 45-49 years and 50-65 years were counted towards both groups. Abbreviations: CNS= central nervous system; OI= opportunistic infections (lymphoma, progressive multifocal leukoencephalopathy, toxoplasmosis, or cryptococcus); HCV= hepatitis C virus; HIV= human immunodeficiency virus; NP= neuropsychological; ART= antiretroviral

therapy; VL= HIV-1 viral load; Heavy drug use= crack, cocaine, or heroin use >50% of visits during study period.

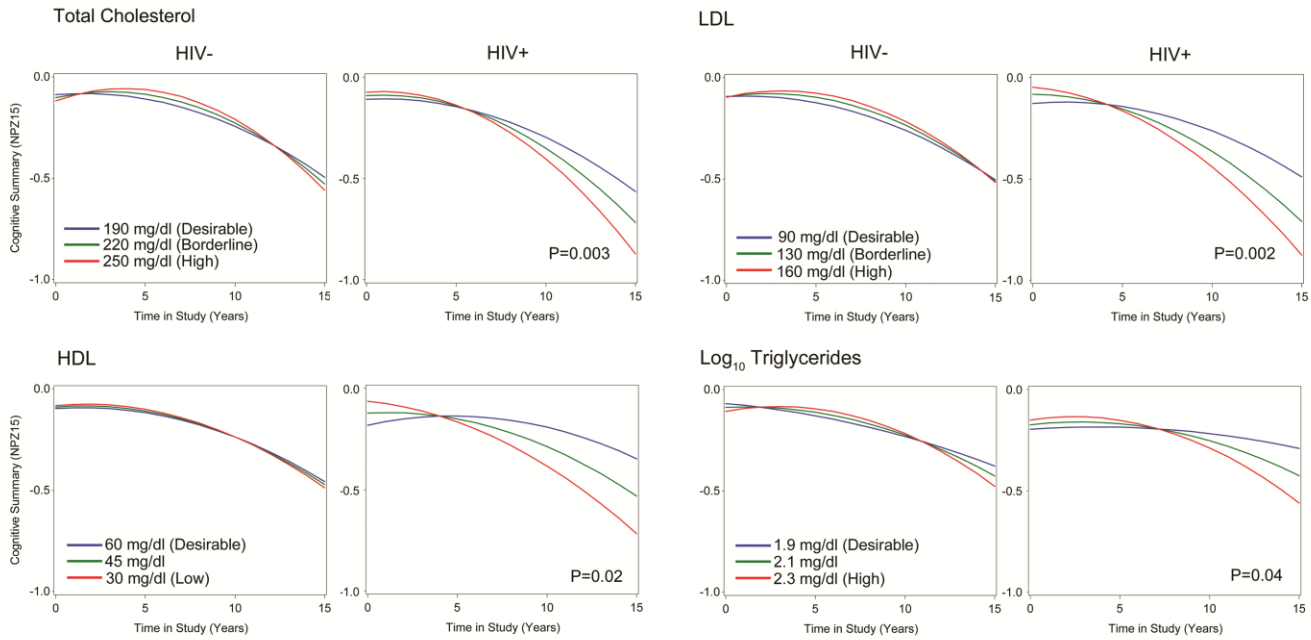


Figure 2: Higher Total Cholesterol, LDL, and Triglycerides are Associated with Faster Rates of Cognitive Decline, While Higher HDL Levels Attenuate Decline in ART-treated HIV+ Men

Estimated slopes in neurocognitive scores according to total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and $\log_{10}$ triglyceride levels stratified by HIV infection, and categorized by National Cholesterol Education Program guidelines are shown. The slopes are estimated for a man with study entry age of 50, and cohort mean IQ score 108, baseline CES-D score 9, and CD4 count held at 800 cells/ml. The x-axis is time in study (years) centered at zero for the first visit after age 50, and y-axis is the change in cognitive performance from the baseline score. There is an accelerated rate of age-related decline in the cognitive score as total cholesterol and triglycerides levels increase in HIV- and HIV+ men, an effect not observed for LDL or HDL levels. Higher total cholesterol (P=0.003), LDL (P=0.002), and triglycerides (P=0.04) levels in HIV+ men are associated with a steeper slope of cognitive decline during the study, while higher HDL levels attenuated the rate of decline (P=0.02).

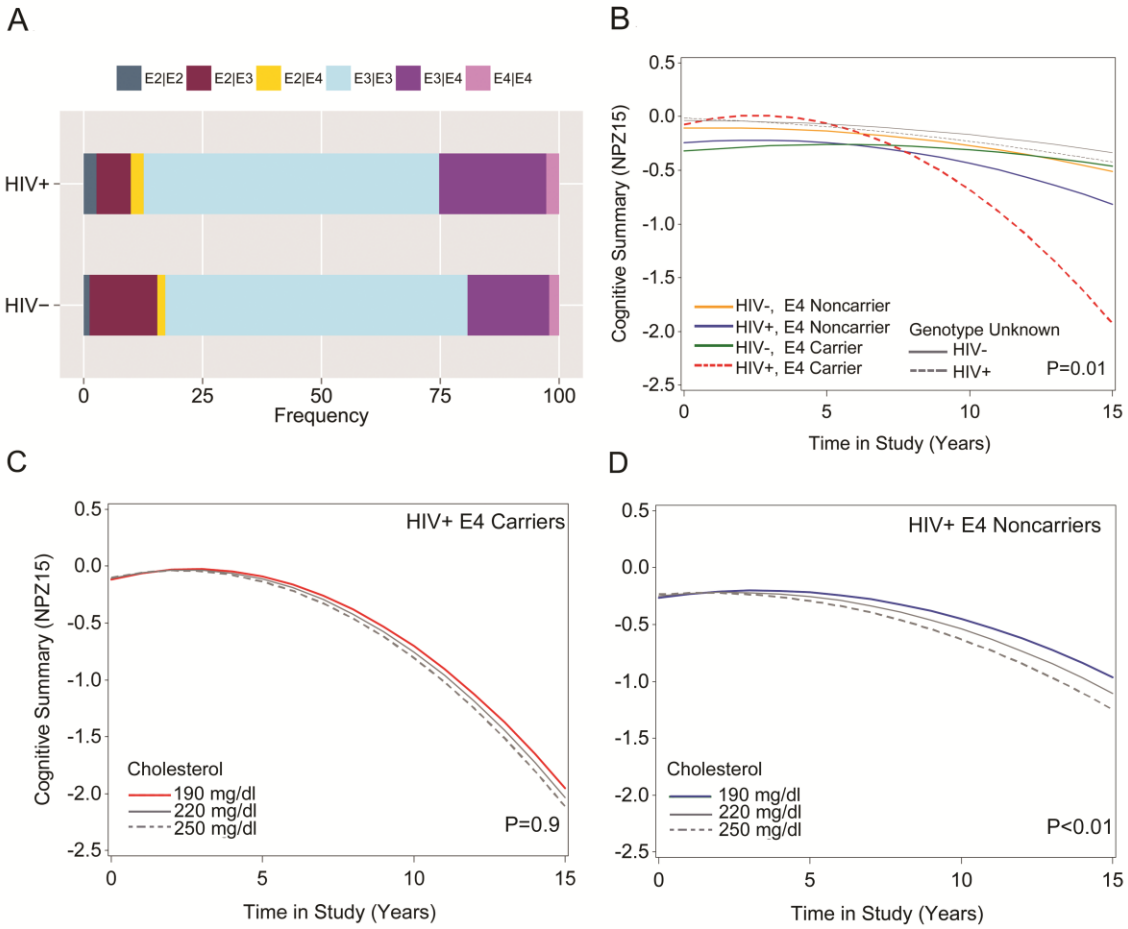
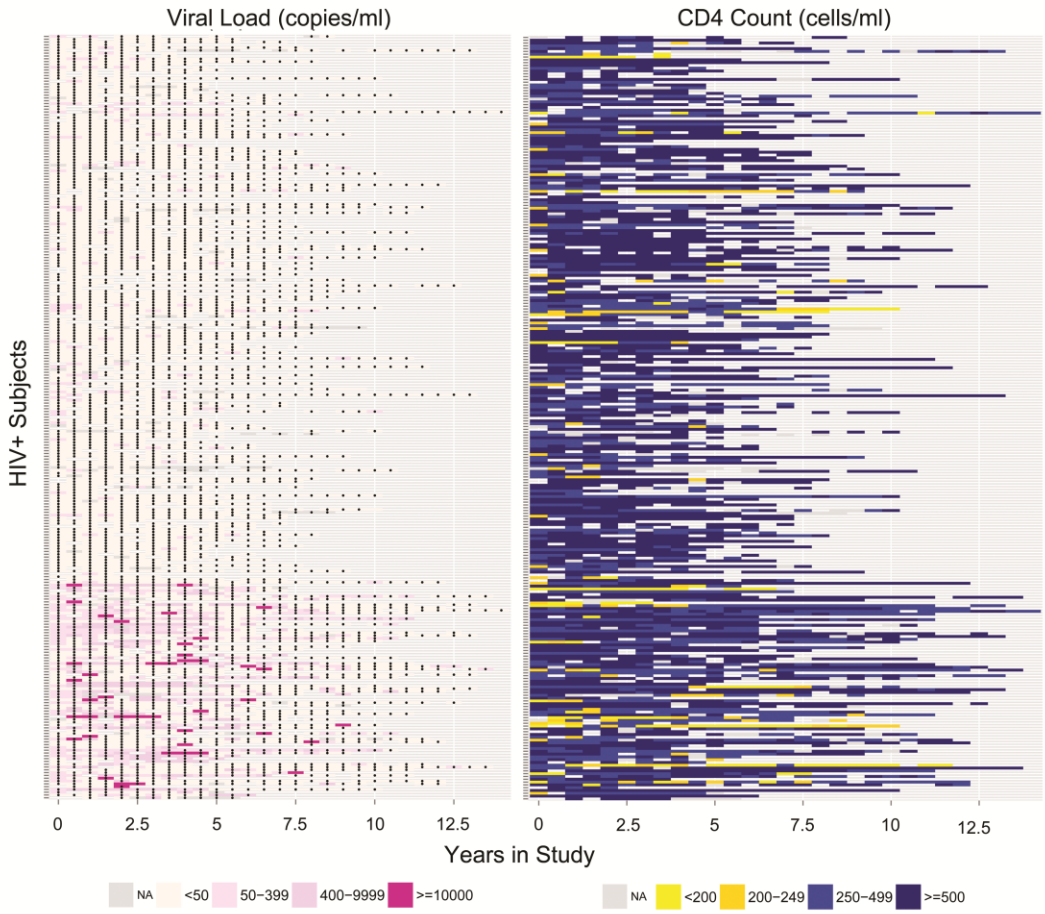


Figure 3: APOE ε4 Allele and Total Cholesterol Have Independent Effects on Cognitive Decline in ART-treated HIV+ Men

The distribution of APOE genotypes among HIV+ and HIV- subjects (A), and estimated slopes for cognitive scores for APOE ε4 allele and HIV infection status are shown (B). Cognitive scores for subjects with unknown or ε2/ε2 genotypes are shown in grey (B). Among those with ε4 genotype, cognitive decline for HIV+ ε4 carriers (C) and noncarriers (D) modified by total cholesterol are shown. The annual rate of decline is estimated for a man with baseline age 50, and cohort mean IQ score 108, baseline CES-D score 9, and CD4 count held at 800 cells/ml. APOE ε4 carriers had lower baseline cognitive scores than noncarriers (P=0.03), an association that was not modified by HIV infection (P=0.14). HIV+ APOE ε4 carriers showed accelerated decline in cognitive scores between ages 50-65 and the rate of accelerated decline was faster than predicted for HIV+ noncarriers (P=0.01). Increasing total cholesterol levels was

associated with faster rates of decline among HIV+ ε4 noncarriers (P<0.01), while the accelerated rate of decline in HIV+ ε4 carriers was not further modified by cholesterol (P=0.9).



Supplementary Figure 1: Longitudinal HIV Plasma Viral Loads and CD4 Counts for 279 HIV+ Subjects

Lasagna plots visualizing HIV-1 plasma viral loads (left panel) and CD4 counts (right panel) for each subject over time. Subjects' person-visits are represented in a single row. The vertical order of the lasagna plots is based on relative viral load suppression, with subjects demonstrating the greatest variability in HIV viral load at the bottom of the plot. Increasing viral load is depicted with increasing color intensity (left). CD4 counts are categorized by relevant clinical cutoffs with bright yellow indicating CD4 <200 cells/ml (right). Grey values represent missing viral load or CD4 count during a study visit where additional data was available; black dots indicate self-reported ART use (left).

Supplementary Data

Methods

Study population details

Details of the study design, demographic characteristics, and enrollment patterns among different recruitment periods (1984-85, 1987-91, 2001-2003) for the MACS have been previously described in detail [49, 50]. All subjects were invited to participate in the NP study at the time of their regular MACS visit irrespective of HIV serostatus or cognitive symptoms until the maximum allowable cohort for that center was recruited. For the present study, exclusion criteria were subjects outside the age of interest (45-65 years old), history of CNS opportunistic infections or CNS lymphoma, self-reported cocaine, crack or heroin use at >50% of visits during the study period, HIV seroconversion date unknown or occurring within the study window, < 2 visits with NP data, and missing data required for matching. Among HIV+ men, additional exclusion criteria were ART nonadherence (defined as self-reported ART use < 95% of study visits), reported not taking ART at more than one study visit, study entry visit VL ≥ 400 copies, and all subsequent visits with VL ≥ 400 copies/ml. CNS penetration effectiveness (CPE) score was calculated for each visit based on the 2010 CPE table [49].

Outlier algorithm and missing data

Outliers on NP tests were defined as having z-scores < -5; if no identifiable reason for an outlying value was determined, values were imputed using the average of NP scores from the prior and subsequent visits within 6 months. When elapsed time recorded for NP tests was greater than the tests' maximum time per instructions, values were imputed to maximum time allowed for the test. A total of 176 person-visits met above criteria and values for these visits were imputed (0.35% of person-visits in the observation period).

Supplementary Table1: List of 15 Cognitive Tests Categorized in Domains[1]

DOMAIN	INDIVIDUAL NEUROCOGNITIVE TESTS
Executive Function	Trail-Making Test Part B Stroop Interference Task
Perceptual Speed	Symbol Digit Modalities Test Stroop Color Naming Stroop Word Naming Trail-Making Test Part A
Verbal Memory -Learning and Retrieval	RAVLT Sum of Trials 1 to 5 RAVLT-Immediate Recall RAVLT-Delayed Recall
Attention/Working Memory	CALCAP-Mean Simple Reaction time (1) and Mean Complex Reaction Time (3,4,14)
Motor/Processing Speed	Grooved Pegboard (both hands)

[1]To reduce floor and ceiling affects, we created summary measures of cognitive function. Briefly, each summary measure was calculated by converting individual tests to z-scores using the baseline mean and SD from a cohort of HIV- and HCV- subjects, ages 45-49 years old without heavy drug use. The signs for some timed tests were changed so that higher z-scores always denote better performance. We averaged z-scores for the 15 tests to create the cognitive summary score with the requirement that all scores be present. The individual domain scores were created in a similar fashion.

Abbreviations: RAVLT= Rey Auditory Verbal Learning Test; CALCAP= California Computerized Assessment Package http://www.calcaprt.com/calcap.htm

Supplementary Table 2: Effect of Total Cholesterol and HIV Infection on the Annual Rate of Cognitive Decline

	Cognitive Summary			Executive Function			Perceptual Speed			Verbal Memory			Attention/Working Memory			Motor Speed		
	β	SE	*P* value	*β*	SE	*P* value	*β*	SE	*P* value	*β*	SE	*P* value	*β*	SE	*P* value	*β*	SE	*P* value
Intercept	-0.8941	0.528	0.091	-1.9961	0.715	0.006	-1.3326	0.637	0.037	-0.772	0.7748	0.320	-0.6395	0.6555	0.330	1.0682	0.8415	0.205
Age at Study Entry	-0.04255	0.008222	<.0001	-0.05695	0.011	<.0001	-0.04907	0.010	<.0001	-0.05134	0.01187	<.0001	-0.02016	0.01004	0.045	-0.07055	0.01311	<.0001
IQ	0.02639	0.002608	<.0001	0.04022	0.003	<.0001	0.03257	0.003	<.0001	0.02945	0.003607	<.0001	0.01808	0.003216	<.0001	0.02215	0.004018	<.0001
CES-D Score at Study Entry	-0.00597	0.002699	0.027	-0.00995	0.003	0.005	-0.01049	0.003	0.001	-0.00039	0.003868	0.920	-0.01033	0.003292	0.002	-0.01192	0.004344	0.006
Smoker	-0.08384	0.06093	0.17	-0.04176	0.082	0.609	-0.06998	0.075	0.349	-0.06537	0.08788	0.457	-0.00827	0.07385	0.911	-0.1856	0.09686	0.056
CD4 Count	0.000277	0.000129	0.033	0.000345	<0.001	0.106	0.000296	<0.001	0.071	0.000404	0.000256	0.114	0.000309	0.000196	0.116	0.000341	0.000269	0.206
CD4 Count * CD4 count	-1.17E-7	0	<.0001	-1.68E-07	0	<.0001	-1.32E-07	0	<.0001	-1.48E-07	0	<.0001	-1.61E-07	0	<.0001	-2.10E-07	0	<.0001
HIV Infection	-0.2217	0.1234	0.073	-0.03788	0.202	0.852	-0.06751	0.158	0.669	-0.2724	0.2479	0.272	-0.5679	0.1841	0.002	-0.348	0.2651	0.190
Total Cholesterol (10mg/dl)	-0.00049	0.000454	0.912	0.01041	0.008	0.025	0.00206	0.006	0.387	-0.0163	0.00972	0.352	-0.0179	0.00702	0.439	-0.0057	0.01034	0.738
Total Cholesterol (10mg/dl) * HIV Infection	0.01058	0.00540	0.051	0.00373	0.009	0.687	0.00303	0.007	0.665	0.0197	0.01157	0.089	0.02793	0.00846	0.001	0.00656	0.01232	0.595
Years in Study	-0.06829	0.03077	0.187	0.03266	0.053	0.486	-0.00799	0.04	0.639	-0.1502	0.06719	0.067	-0.1452	0.04948	0.064	-0.1407	0.07149	0.081
Years in Study * Years in Study	0.003250	0.002771	0.241	-0.00167	0.005	0.725	-0.00291	0.004	0.412	0.01802	0.006033	0.003	0.003198	0.00451	0.479	0.003278	0.006391	0.608
HIV Infection * Years in Study	0.06134	0.02256	0.007	0.002474	0.039	0.949	0.05021	0.03	0.088	0.07245	0.04812	0.133	0.1194	0.03533	0.001	0.0504	0.05298	0.342
Total Cholesterol (10 mg/dl) * Years in Study	0.00398	0.00155	0.112	-0.0002	0.003	0.970	0.00064	0.002	0.775	0.00874	0.00339	0.043	0.00618	0.00252	0.191	0.00571	0.00036	0.220
Total Cholesterol (10 mg/dl) * Years in Study * Years in Study	-0.0003	0.00014	0.043	-6.94E-05	<0.001	0.777	3.88E-05	<0.001	0.832	-0.0009	0.00031	0.003	-0.0002	0.00024	0.390	-0.0004	0.00033	0.202
Total Cholesterol (10 mg/dl)* HIV serostatus * Years in Study	-0.0034	0.00112	0.003	0.00017	0.002	0.930	-0.0023	0.001	0.110	-0.0047	0.00241	0.051	**-0.0062**	**0.00178**	**0.001**	-0.0032	0.00263	0.217

Mixed models were adjusted for age at study entry, Shipley WAIS IQ- Equivalent Score, CES-D at study entry, smoking status, CD4 count (linear and quadratic term). Cholesterol estimates were interpreted in 10 mg/dl increments. A negative estimate indicates a steeper slope of cognitive decline

Abbreviations: CES-D= Center for Epidemiological Studies Depression Scale, IQ =Shipley WAIS IQ- Equivalent Score, β=Estimate, SE= standard error, * indicates an interaction

Supplementary Table 3: APOE Clinical Characteristics

	All Subjects	HIV- E4 NonCarrier	HIV- E4 Carrier	HIV+ E4 Noncarrier	HIV+ E4 Carrier	*P* value	*P* value between HIV+ noncarrier vs. HIV+ carrier	*P* value between noncarrier vs. carrier
No. of patients	344	186	50	77	31	NA		
Length of Followup, mean (SD), years	7.2 (3.0)	6.8 (2.7)	6.8 (3.3)	7.7 (3.0)	6.7 (3.3)	0.13	0.13	0.3844
Demographics								
Baseline Age, median (Q1-Q3) years	50.0 (50-54)	51.0 (50-54)	51.0 (50-53)	50.0 (50-51)	50.5 (50-54)	0.03	0.0684	0.9357
Race/Ethnicity, No. (%)								
White	292 (84.9)	165 (88.7)	43 (86.0)	57 (74.0)	27 (87.1)	0.102		
Black	38 (11.1)	12 (6.5)	7 (14.0)	16 (20.8)	3(9.7)			
Hispanic or Latino	9 (2.6)	5 (2.7)	0	3(3.9)	1 (3.2)			
Other	5 (1.5)	4 (2.2)	0	1 (1.3)	0			
Education >12 years, No. (%)	344 (100)	186 (100)	50 (100)	77 (100)	33 (100)	NA		
Shipley WAIS IQ- Equivalent, mean (SD)	109.5 (8.9)	111.2 (7.7)	107.1 (9.4)	107.3 (10.0)	110.0 (9.2)	0.09	0.22	0.3475
Depression Profile								
Baseline CES-D Score, mean (SD)	8.2 (9.1)	7.8 (8.7)	6.9 (10.2)	9.6 (9.4)	9.5 (9.1)	0.18	0.99	0.6237
Baseline CES-D Score ≥ 16, No. (%)	60 (17.4)	31 (16.7)	7 (14.0)	15 (19.5)	7 (21.2)	0.22	0.5616	0.9584
Substance Use, No. (%)								
Smoking (Highest use on ≥ 2 visits)						0.7		
None	270 (78.5)	148 (79.6)	35 (70.0)	61 (79.2)	26 (83.9)			
<1/2 pack per day	30 (8.7)	14 (7.5)	6 (12.0)	7 (9.1)	3 (9.7)			
1/2 - 2 packs	44 (12.8)	24 (12.9)	9 (18.0)	9 (11.7)	2 (6.5)			
Alcohol (Highest use on ≥ 2 visits)[1]						0.62		
None	47 (13.7)	27 (14.5)	6 (12.0)	9 (11.7)	5 (16.1)			
Occasional-Moderate	257 (74.7)	143 (76.9)	37 (74.0)	55 (71.4)	22 (71.0)			
Heavy/Binge	40 (11.6)	16 (8.6)	7 (14.0)	13 (16.9)	4 (12.9)			
Baseline Lipid Profile, Mean (SD)								
Total Cholesterol	199.7 (36.2)	199.3 (34.2)	193.5 (30.6)	199.6 (41.5)	212.8 (41.3)	0.56	0.2009	0.8536
LDL	119.6 (33.1)	122.0 (32.8)	121.4 (49.7)	115.6 (35.8)	115.0 (34.1)	0.37	0.9455	0.7329
HDL	47.8 (13.3)	48.1 (12.2)	48.6 (12.9)	45.8 (11.4)	49.3 (23.0)	0.53	0.4861	0.4307
Triglycerides	142.6 (96.2)	128.3 (76.2)	121.4 (49.7)	151.4 (71.5)	285.8 (216.8)	<0.001	<0.001	0.05
Lipid-lowering Medication, No. (%)[3]								
Statin	143 (41.6)	64 (34.4)	21 (42.0)	40 (51.9)	18 (58.1)	0.013		
Fibrate	34 (9.9)	6 (3.2)	5 (10.0)	14 (18.2)	9 (29.0)	<0.001		
Niacin	19 (5.5)	11 (5.9)	2 (4.0)	5 (6.5)	1 (3.2)	0.867		
HCV Antibody Positive, No. (%)	16 (4.7)	5 (2.7)	3 (6.0)	6 (7.8)	2 (6.5)	0.003	0.5847	0.545
HIV Disease Characteristics, Median (Q1-Q3)								
Baseline CD4 (cells/ml)[2]	859 (604-1104)	948 (771-1153)	1072 (777-1268)	536 (346-743)	457 (331-630)	<0.001	0.4821	0.846
CD4 nadir (cells/ml) in observation	647 (458-862)	718 (575-1100)	804 (595-967)	387 (228-553)	407 (287-624)	<0.001	0.5638	0.9913
Baseline Log_{10} HIV RNA VL (copies/ml)[2]				1.6 (1.6-1.6)	1.6 (1.6-1.6)	0.72	NA	NA
Baseline CPE Score[2]				7.0 (6-9)	8.0 (6-9)	0.26	NA	NA
Antiretroviral Medication, No. (%)[3]								
Azidothymidine				28 (36.4)	9 (29.0)	0.51	NA	NA
Efavirenz				33 (42.9)	20 (64.5)	0.056	NA	NA
Protease Inhibitor				56 (72.7)	18 (58.1)	0.171	NA	NA

ddI, d4T, ddC	34 (44.2)	13 (41.9)	1	NA	NA
Abacavir	29 (37.7)	17 (54.8)	0.132	NA	NA

P values <0.05 were considered significant.

1 Alcohol Use: Light= <1 drink/week; Occasional-Moderate= 1-14 drink(s)/week; Heavy= >14/week, Binge= ≥5 drinks/one sitting/month

2 Baseline values: first visit or within 6 months of study period

3 Self-reported Lipid-lowering or ART medication used on ≥ 2 visits

Abbreviations: SD= standard deviation; Q1-Q3: Quartile1 – Quartile3; NA= not applicable; CES-D= Center for Epidemiological Studies Depression Scale; LDL= low-density lipoprotein;

HDL=high-density lipoprotein; HCV= hepatitis C virus; HIV= human immunodeficiency virus; VL= HIV-1 viral load; CPE= CNS Penetration Effectiveness; ddI= Didanosine; d4T= Stavudine; ddC=Zalcitabine

Supplementary Table 4: Effect of the APOE E4 allele and HIV Infection on Annual Rate of Cognitive Decline

	Cognitive Function			Executive Function			Perceptual Speed			Verbal Memory			Attention/Working Memory			Motor Speed		
	β	**SE**	***P* value**	***β***	**SE**	***P* value**	***β***	**SE**	***P* value**	***β***	**SE**	***P* value**	***β***	**SE**	***P* value**	***β***	**SE**	***P* value**
Intercept	-1.0077	0.5332	0.060	-1.8938	0.7121	0.008	-1.3487	0.6426	0.036	-0.8461	0.7612	0.267	-1.0148	0.6471	0.118	0.9204	0.826	0.266
Age at Study Entry	-0.0426	0.0026	<.0001	-0.05531	0.01106	<.0001	-0.04861	0.01022	<.0001	-0.05508	0.003592	<.0001	-0.01867	0.01005	0.064	-0.0701	0.01315	<.0001
IQ	0.0268	0.0083	<.0001	0.04133	0.003405	<.0001	0.03314	0.003055	<.0001	0.02947	0.01198	<.0001	0.01734	0.003194	<.0001	0.02204	0.00398	<.0001
CES-D Score at Study Entry	-0.0062	0.0027	0.021	-0.00949	0.003533	0.007	-0.00944	0.003267	0.004	-0.00042	0.003838	0.914	-0.01126	0.003275	0.001	-0.01213	0.004295	0.005
Smoker	-0.1198	0.0602	0.047	-0.08532	0.07985	0.286	-0.1235	0.07356	0.094	-0.1042	0.08598	0.226	-0.03051	0.07255	0.674	-0.1923	0.09431	0.042
CD4 Count	0.0003	0.0001	0.021	0.00032	0.000194	0.100	0.000176	0.000151	0.244	0.000329	0.000239	0.169	0.000361	0.000183	0.048	0.000377	0.000245	0.125
CD4 Count * CD4 count	-1.10000E-07	0	<.0001	-1.37E-07	0	<.0001	-6.63E-08	0	<.0001	-1.09E-07	0	<.0001	-1.83E-07	0	<.0001	-2.16E-07	0	<.0001
HIV Infection	0.0236	0.0829	0.539	0.05827	0.1157	0.373	0.1088	0.1026	0.803	0.159	0.1309	0.005	-0.03028	0.107	0.907	-0.1296	0.1404	0.274
APOE E4 carrier	-0.2834	0.1352	0.032	-0.5901	0.1764	0.000	-0.4019	0.1591	0.003	-0.482	0.1952	0.288	0.05679	0.163	0.236	-0.1837	0.2078	0.593
HIV Infection * APOE E4 carrier	0.2259	0.1953	0.139	0.3937	0.2615	0.031	0.11	0.2361	0.037	0.6559	0.2887	0.025	0.09321	0.2362	0.872	0.0968	0.3093	0.788
Years in Study	0.0002	0.0110	0.014	-0.01803	0.01857	0.000	-0.01272	0.01429	0.004	0.03843	0.02432	0.565	-0.03702	0.01758	0.157	-0.02473	0.0252	0.077
Years in Study * Years in Study	-0.0013	0.0011	<0.001	0.00064	0.001868	<.0001	-0.00085	0.001433	<.0001	-0.00311	0.00245	0.603	0.000789	0.001809	0.098	-0.00537	0.002528	<.0001
Years in Study * HIV Infection	-0.0104	0.0168	0.266	0.02892	0.02837	0.017	-0.00734	0.02171	0.137	-0.04124	0.03678	0.021	-0.00778	0.02722	0.099	-0.04815	0.03828	0.452
Years in Study * Years in Study * HIV Infection	0.0002	0.0016	0.003	-0.0023	0.002745	0.001	0.001083	0.002096	0.014	0.003141	0.003581	0.233	-0.00045	0.002669	0.030	0.003612	0.003707	0.872
Years in Study * APOE E4 Carrier	0.0227	0.0243	0.022	0.05367	0.0413	0.003	0.06773	0.03153	0.000	0.0252	0.0537	0.507	-0.02599	0.03992	0.032	0.02425	0.05579	0.540
Years in Study * Years in Study * APOE E4	-0.0008	0.0022	0.002	-0.00157	0.00393	0.010	-0.00376	0.002986	0.000	-0.00134	0.005148	0.235	0.003692	0.003769	0.020	-0.0013	0.005268	0.288
HIV Infection * APOE E4 * Years in Study	0.0519	0.0355	0.300	0.02545	0.05972	0.474	0.0538	0.04564	0.277	-0.04608	0.07666	0.758	0.09662	0.05621	0.221	0.03467	0.08193	0.775
HIV Infection * APOE E4 * Years in Study * Years in Study	**-0.0106**	**0.0035**	**0.010**	-0.00848	0.005731	0.212	**-0.01095**	**0.00438**	**0.035**	-0.00029	0.007365	0.981	-0.00998	0.005382	0.179	-0.01001	0.008017	0.459

P values <0.05 were considered significant

Abbreviations: APOE= apolipoprotein E, CES-D= Center for Epidemiological Studies Depression Scale, IQ =Shipley WAIS IQ- Equivalent Score, SE= standard error,

* indicates an interaction, Bold indicates statistical test of interest

Supplementary Table 5: Effect of Total Cholesterol and APOE on Annual Rate of Cognitive Decline

	Neurocognitive Score		
	β	**SE**	**Pvalue**
Intercept	-0.5435	0.8214	0.509
IQ	0.03346	0.004369	<.0001
Baseline Age	-0.06233	0.01233	<.0001
Baseline CES-D Score	-0.00628	0.003774	0.0972
Smoker	0.01083	0.08889	0.9032
APOE4 carrier	-0.2	0.2395	0.9812
CD4	0.000098	0.000196	0.6158
CD4*CD4	-3.63E-08	0	<.0001
HIV Infection	-0.2988	0.2171	0.6371
Total Cholesterol (10 mg/dl)	-0.0011	0.00634	0.8783
Total Cholesterol (10 mg/dl) * HIV+ infection	0.00597	0.00948	0.5295
APOE4 carrier * HIV+ infection	0.3895	0.197	0.0495
Total Cholesterol (10 mg/dl) * APOE4 carrier	-0.0023	0.00937	0.8094
Years in Study	-0.0418	0.02907	0.2574
Years in Study * Years in Study	-0.00175	0.001143	<.0001
Years in Study * APOE4 carrier	-0.02388	0.06127	0.8136
Years in Study * HIV Infection	0.1452	0.04568	0.0005
Years in Study * Total Cholesterol (10 mg/dl)	0.00214	0.00125	0.8417
Years in Study * Total Cholesterol (10 mg/dl) * HIV infection	-0.0056	0.00184	0.0021
Years in Study * APOE4 carrier * HIV infection	0.0266	0.07136	0.71
Years in Study * Years in Study * APOE4 carrier * HIV infection	-0.01099	0.003421	0.0058
Years in Study * APOE4 carrier * Total Cholesterol (10 mg/dl)	0.00192	0.00252	0.3598
Years in Study * APOE4 carrier * HIV Infection * Total Cholesterol (10 mg/dl)	**-0.00004**	**0.000308**	**0.897**

P values <0.05 were considered significant

Abbreviations: APOE= apolipoprotein E, CES-D= Center for Epidemiological Studies Depression Scale, IQ =Shipley WAIS IQ- Equivalent Score, β=Estimate, SE= standard error, * indicates an interaction, Bold indicates statistical test of interest

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CONFLICTS OF INTEREST

The authors reports no conflicts of interest

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Chapter Ib:

Preliminary Studies on the Association between Statin Medications and Midlife Cognitive Decline in ART-Adherent HIV+ Men

(This work has not been submitted for publication)

Objective

Hyperlipidemia or dyslipidemia is a pathologic cardiometabolic condition with important relevance for cognitive function (reviewed [51]). In the Cardiovascular Risk Factors Aging and Dementia (CAIDE) study, midlife total cholesterol predicted cognitive impairment 21 years later [19], and in a population-based study from the Kaiser Permanente Medical Care Program, high midlife cholesterol level increased the risk for Alzheimer's Disease and Vascular Dementia [52]. While cholesterol-lowering agents such as statins are able to protect against stroke as shown in cohort studies, and the clinical trial, Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL)[53, 54], this has not translated to improved cognitive function in the general population [47].

Statins have anti-inflammatory properties that include reduction of reactive oxygen species, and endothelial nitric oxide synthase. Statin treatment of moncytes reduces CD11β expression and inhibits cellular adhesion to the endothelium [55]. In animal studies, the number of leukocytes in atherosclerotic plaques is reduced with statin treatment even in the absence of effects on plasma lipid levels [56]. In chapter Ia, we showed that increasing cholesterol levels were associated with faster rates of cognitive decline in ART-adherent HIV+ men between ages 50-65 years, an effect not observed in HIV- controls. Emerging data from cohort-studies suggest that HIV+ individuals are at increased risk for cardiovascular disease, arterial inflammation assessed by positron emission tomography with 18fluorine-2-deoxy-D-glucose and high-risk atherosclerotic plaques compared to Framingham risk score-matched controls [57, 58]. It is possible that statins therapy may attenuate the rate of midlife cognitive decline among HIV+ men through reduction in inflammation, atherosclerosis, cholesterol levels and/or yet unidentified mechanisms.

In this section, we present preliminary data based on the methods described in chapter 1a to determine if cognitive decline is influenced by statin use in ART-adherent HIV+ men between ages 50-65 years, and whether statin therapy and total cholesterol levels interact to influence cognitive decline in HIV- infection.

Statistical Methods

The association between statin use, HIV infection, and change in cognitive score was examined using mixed-effects models with interaction terms for statin use with time, HIV infection with time, and their joint interaction with time; statin use was analyzed as a dichotomous (statin vs. no statin), time-varying covariate. Continuous variables included baseline age at study entry, CES-D score, and IQ score; CD4 cell count was examined as a time-varying covariate. Smoking and HIV infection were analyzed as binary covariates. A backwards elimination procedure was used to identify significant longitudinal relationships among predictors (P <0.05 cut-off). Random predictors were correlated intercepts and linear slopes of time. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Preliminary Results and Interpretation

HIV+ men were more likely to report being on statin therapy than HIV- controls during a study visit (37% vs. 23% of total visits/group; data not shown). A total of eight statin medications were reported; Simvastatin was used alone or in combination with Ezetimibe (Figure 1). Lipitor was the most commonly used medication in both groups (61%); Pravastatin and Simvastatin were the second most common statin therapies among HIV+ and HIV- subjects, respectively.

Statin Use, Total Cholesterol and Cognitive Decline

Time-related terms reflecting the association between statin use, and cognitive decline are summarized in Table 1. There was no significant relationship between statin use, and rate of decline in HIV+ men (p=0.40); the interaction in HIV- subjects was marginally significant (p=0.06). Figure 2 illustrates these findings for a representative 50 year old man with or without HIV infection. In a separate mixed-effects model that included terms for HIV infection, total cholesterol, statin use and their respective interactions with time, cholesterol level independently influenced the rate of cognitive decline in HIV+ men, corroborating results from chapter 1a (estimate= -0.00052U/yr; p= 0.02). The association between statin use and cognitive decline in HIV+ men was marginally significant (p=0.05). When the interaction between HIV infection, statin use, total cholesterol and time was tested, statin use substantially modified the association between total cholesterol and the annual rate of decline in HIV+ individuals (p=0.015; Figure 2 lower panel). These findings suggest that in HIV+ men between ages 50-65 years, total cholesterol levels are independently associated with faster rates of cognitive decline, and statins

potentially influence cognitive decline through interactions with total cholesterol. These are preliminary studies, and further work is needed to better understand the clinical cohort on statin therapy, the relationship between statin use and other lipid markers, and the influence of APOE E4 genotype on these respective associations.

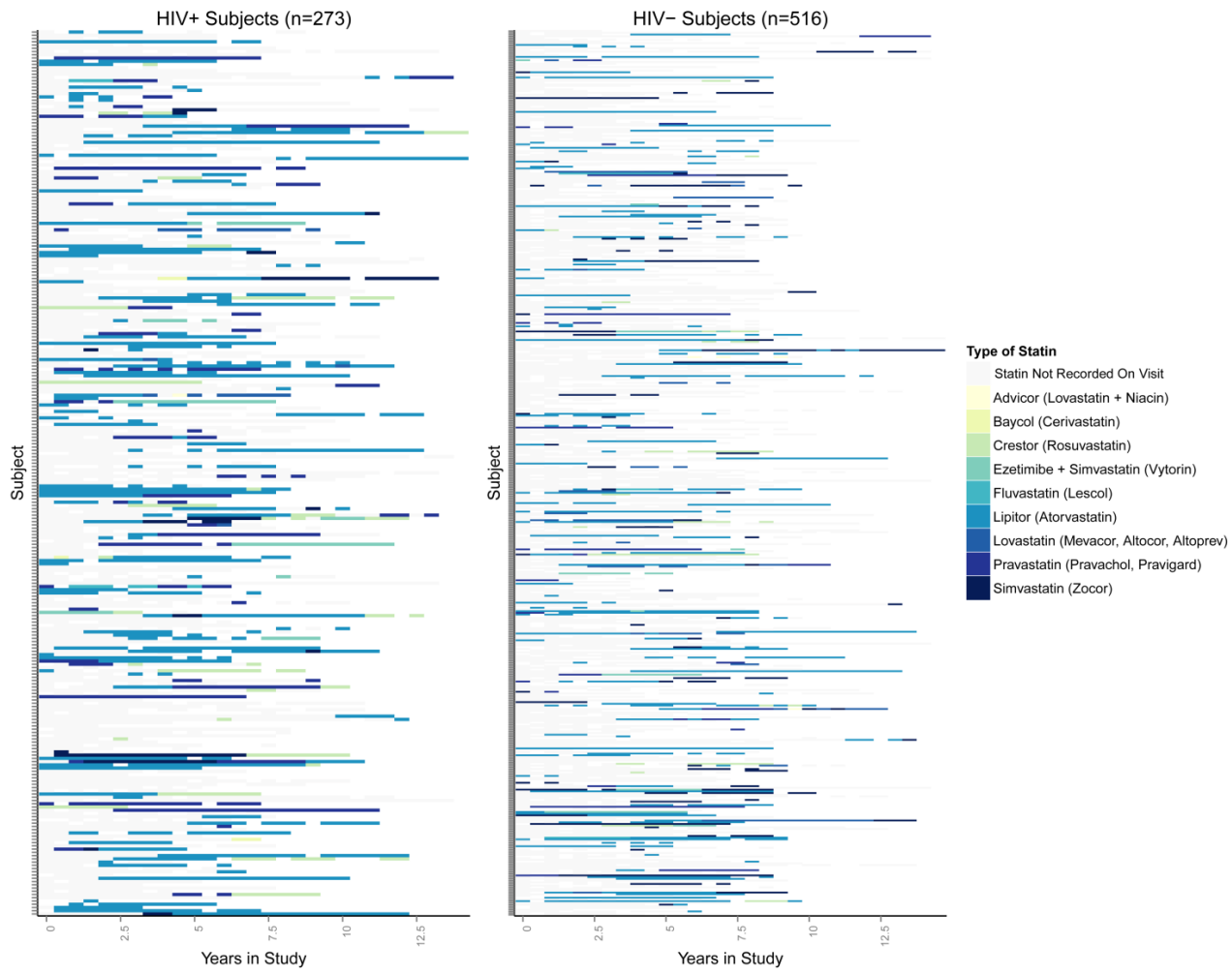

Figure 1: Statin Use in 279 ART-Adherent HIV+ and 516 HIV- Men Enrolled in the MACS

Lasagna plots visualizing type of statin medication reported for each HIV+ (left) and HIV- (right) subject. Subjects' person-visits are represented in a single row. The vertical order for the lasagna plots among HIV+ subjects is based on relative viral load suppression, with subjects demonstrating the greatest variability in HIV viral load at the bottom of the plot. Grey values represent a study visit where additional data was available but statin use was not recorded.

Table 1: Associated Effect of Statin Use and Total Cholesterol on the Annual Rate of Cognitive Decline

Model 1:	**Estimate**	**SE**	***P* value**
HIV+ * Years in Study	-0.0132	0.0071	0.303
Statin Use * Years in Study	0.0073	0.0103	0.060
Statin Use * HIV+ * Years in Study	**0.0118**	**0.0140**	**0.400**
Model 2:			
HIV+ * Years in Study	0.0913	0.0298	0.322
Statin Use * Years in Study	0.0286	0.0287	0.995
Statin Use * HIV+ * Years in Study	**-0.0753**	**0.0374**	**0.050**
Total Cholesterol (10 mg/dl) * Years in Study	0.0014	0.0001	0.368
Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**-0.0052**	**0.0001**	**0.018**
Total Cholesterol (10 mg/dl) * Statin Use * Years in Study	-0.0010	0.0014	0.184
Statin Use *Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**0.0045**	**0.0002**	**0.015**
Model 2:			
HIV+ * Years in Study	0.0913	0.0298	0.322
Statin Use * Years in Study	0.0286	0.0287	0.995
Statin Use * HIV+ * Years in Study	**-0.0753**	**0.0374**	**0.050**
Total Cholesterol (10 mg/dl) * Years in Study	0.0014	0.0001	0.368
Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**-0.0052**	**0.0001**	**0.018**
Total Cholesterol (10 mg/dl) * Statin Use * Years in Study	-0.0010	0.0014	0.184
Statin Use *Total Cholesterol (10 mg/dl) * HIV+ * Years in Study	**0.0045**	**0.0002**	**0.015**

Adjusted for age at study entry, Shipley WAIS IQ- Equivalent Score, CES-D at study entry, smoking status, and CD4 count. Total cholesterol was interpreted in 10 mg/dl increments. Model 1 includes terms for statin use. Model 2 includes terms for both statin use and total cholesterol.
Model 1: R2 for fixed effects= 0.23, P<0.001; Model 2: R2 for fixed effects= 0.25, P<0.001
The squared Pearson correlations of predicted values from fixed versus actual values of the dependent variable indicate the percent of variance accounted for by predictors. A negative estimate indicates a faster rate (steeper slope) of cognitive decline.

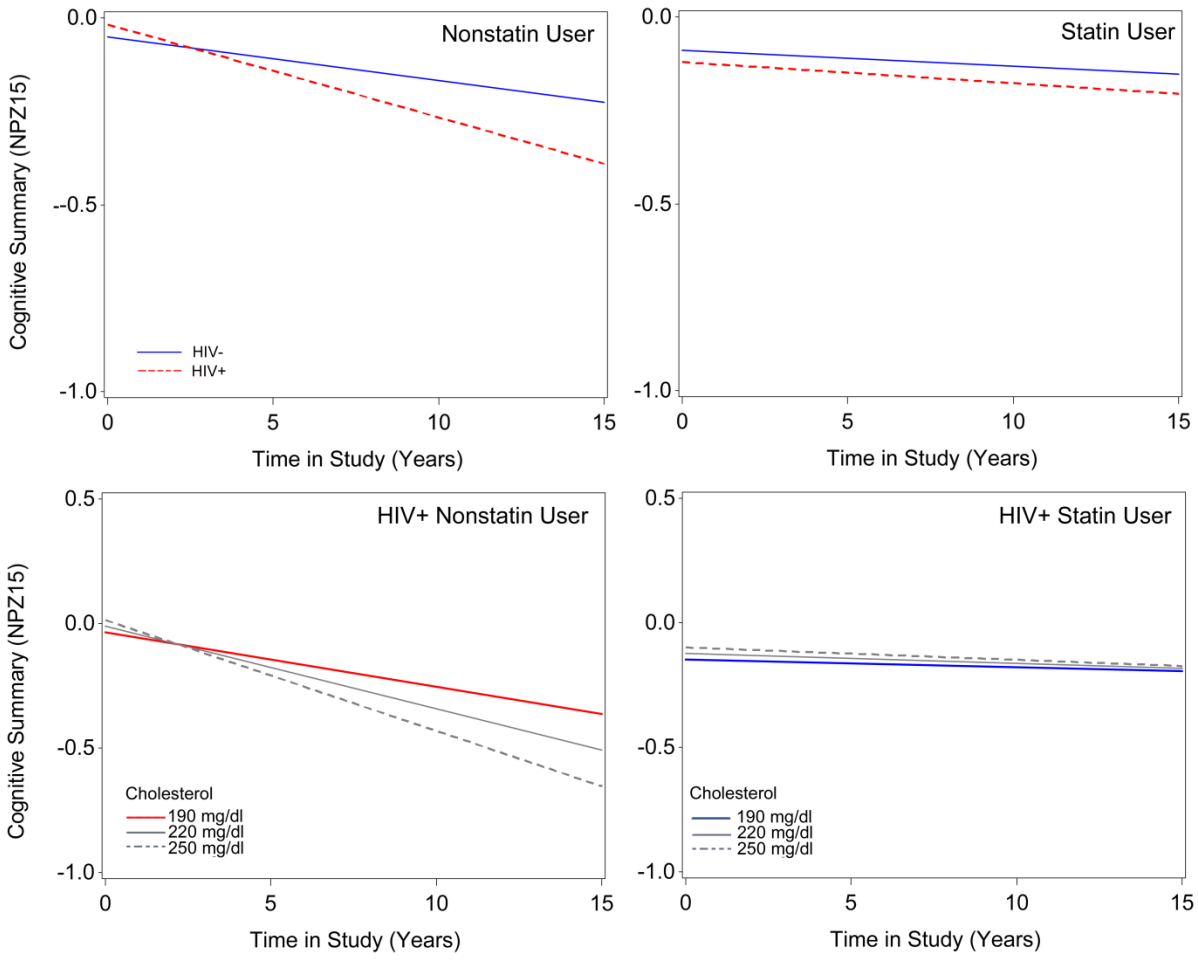

Figure 2: The Association between Total Cholesterol Levels and the Annual Rate of Midlife Cognitive Decline is Modified by Statin Use in ART-adherent HIV+ Men

Estimated slopes in neurocognitive scores according to time-varying statin use, and stratified by HIV infection are shown (top panel); the association between statin use, and cognitive decline in HIV+ men modified by total cholesterol is shown in the lower panel. The slopes are estimated for a man with study entry age of 50, and cohort mean IQ score 108, baseline CES-D score 9, and CD4 count held at 800 cells/ml. The x-axis is time in study (years) centered at zero for the first visit after age 50, and y-axis is the change in cognitive performance from the baseline score. When investigated without adjustment for total cholesterol, there is no association between statin use, and the annual rate of cognitive decline

among HIV+ men (0.4). In a separate mixed-effects model, higher total cholesterol was independently associated with a steeper slope of cognitive decline among HIV+ individuals (p= 0.02), and the rate of decline was substantially attenuated by statin use (p=0.015). This suggests that cholesterol levels independently influences the rate of cognitive decline in HIV+ men between ages 50-65 years, and statin use modifies this association.

CHAPTER II:

Predictors of Age-Related Cognitive Decline in Men with and without HIV-Infection

A. Objective:

The relative contributions of the APOE ε4 genotype, systemic inflammation and CVD on cognitive decline among older ART-adherent HIV+ adults have not been established. The proposed research leverages resources from our cohort of 789 ART-adherent HIV+ and HIV- adults from the longitudinal prospective Multicenter AIDS Cohort Study (MACS) in chapter 1 to determine the extent to which inflammatory markers, CVD risk factors, and APOE ε4 genotype account for cognitive decline among HIV+ adults on ART. These aims utilize longitudinal cognitive modeling to examine the contribution of time-varying laboratory indices predictive of cognitive trajectories, determine the extent to which decline is attributable to these processes, and estimate change-points that signal the onset of cognitive impairment in HIV- and HIV+ subjects. Together, these studies will help address whether common causes of dementia in the general population explain individual differences in cognitive decline among HIV-infected adults over age 50 years, and have potential translational implications for the development of therapeutic targets to prevent brain aging in HIV-infection and other neurodegenerative diseases.

Aim 1: Examine the contribution of inflammatory markers, cardiovascular disease risk factors, and APOE ε4 genotype to the rate of cognitive decline in midlife HIV+ individuals.

In the aim, we will test the hypothesis that circulating inflammatory markers (e.g. hsCRP, IL-6, TNF-α), CVD risk factors (hyperlipidemia, cigarette use, diabetes mellitus, and hypertension), and APOE ε4 genotype (carrier vs. noncarrier) account for >50% of individual differences in the rates of cognitive decline among midlife ART-suppressed HIV+ adults. Using mixed-effects models, we will determine the influence of the above variables on the rate of cognitive decline on composite cognitive score, and in cognitive domains (i.e. verbal memory, processing speed, executive, motor) with directed comparisons between ART-adherent HIV+ subjects and matched HIV-controls ages 50-65. First, to estimate the contribution of individual variables to the rate of decline, we will add in terms for the variable and its interaction with time in a series of models, as described [59]. The estimate for the variance in random time will capture how individual deviate from the mean slopes; thus, the time-by-variable term will account for a proportion of the total random slope variance, and the reduction in the random slope variance will reflect between-subject variation in cognitive decline. In subsequent analyses, significant

variables will be considered simultaneously to determine the additive effect, and cumulative influence on between-subject variation in cognitive decline.

Aim 2: Model change points to estimate when conversion to cognitive impairment occurs in ART-adherent HIV+ individuals. The goal of this aim is to divide cognitive paths into slow change and more rapid change components, and determines when significant variables identified in Aim 1 influence the rate of decline in ART-suppressed HIV+ and HIV- adults over age 50 years. We will fit a series of mixed-effects models that allow the rate of cognitive decline to change in the last n years before cognitive impairment (SD ≤ 1.0, two cognitive domains), and identify analyses with the best fit for the change point (time when rate of decline increases), as described [60]. We will then determine the effect of pathological indices in *Aim 1* on the rate of decline at pre- and post-change point. Analysis will be subsequently repeated using measures of specific cognitive functions in place of the composite cognitive score (impairment defined as SD ≤ 1.5, single cognitive domain).

B. Research Approach:

Subject populations: Subjects are drawn from the MACS, a multicenter prospective study conducted in Baltimore, Chicago, Pittsburgh, and Los Angeles of men who have sex with men [61-64]. Participants were evaluated at 6-month intervals for medical history (including cigarette use), medications including ART and structured neurocognitive testing. Blood/urine specimens were used to test for HIV antibody, T-cell subsets, HIV viral load, HCV serology/plasma RNA, endocrine (fasting glucose, HOMA-IR, HgA1C), lipid (total cholesterol, LDL, HDL, triglycerides), inflammatory markers and APOE genotyping. The Gabuzda lab created a local SQL database containing the entire public dataset streamlined for analysis. All subjects were enrolled with written informed consent and IRB approval and healthy plasma/CSF samples obtained from Bioreclamation with IRB approval from Dana-Farber Cancer Institute.

Longitudinal neurocognitive analysis: MACS longitudinal neurocognitive variables were: Trail Making Test–Parts A and B[65]; Stroop Color/Word Interference Test ([66, 67]); Grooved Pegboard [68]; Symbol Digit Modalities Test[69]; California Computerized Assessment Package (CalCAP[70]); Rey Auditory Verbal Learning Test[71-73]. Scores from 15 of these tests will be converted to z-scores using the mean

and SD from HIV- men ages 45-49 years old, and z-scores will be averaged to yield a composite measure of cognition. Z-scores are used in order to place scores from diverse cognitive tests on a common scale so that they could be combined with approximately equal weighting; further, the use of a composite score has the advantage of minimizing floor and ceiling effects, and is preferable for studies examining change in cognition over many years [59].

Subject selection and statistical/bioinformatic analysis: Cohorts include HIV-vs. HIV+ on suppressive ART ages 50-65 (see chapter 1). Exclusions include CNS related opportunistic infections, lymphoma, and heavy drug abuse. Cohorts will be matched by baseline age, race, smoking, education and alcohol use using the R program MATCHIT. *Aim1:* Mixed-effects models will be used to determine rate of change in domain-specific and global cognitive function over time. We will first begin with an unadjusted model with only the term for time; time is defined as the time (in years). The estimate for time (the slope) corresponds to the mean rate of decline in cognition, and the estimate for the variance in random slope captures how individuals deviate from the mean slopes [59]. We will add in terms for HIV-infection, other relevant HIV-disease characteristics, and for the variables of interest and their interactions with time in a series of models. *Aim2:* Fixed change point analysis will be used to identify acceleration in the mean rate of decline in domain-specific and global cognitive outcome measures as described [60]. In brief, we will construct a series of mixed-effects models that allow the rate of cognitive decline to change in the last n years before cognitive impairment. We will test values of n ranging from 0.5 to 3 years, and the distribution of follow-up time in this cohort. Best fit will be defined as analysis with the highest log likelihood.

Power calculations: We identified a cohort of 789 HIV- and ART-adherent HIV+ from the MACS. A power calculation estimates we require minimum sample size of 90 subjects in each group to detect a standardized mean difference of 0.3 with an 85% power using a two-sided 0.05 Wilcoxon test. This supports the proposed dataset is powered to detect differences without inclusion of further subjects.

Conclusions

Aging is characterized by progressive cognitive decline, and the prevention of cognitive impairment is a major public health challenge in the 21st century [12, 59, 60]. Superimposed effects of HIV-associated inflammation and oxidative injury may increase the rate of cognitive decline among a subset of ART-suppressed HIV+ individuals over age 50 years. Cognitive decline in old age regardless of HIV serostatus is a complex phenomenon, and there is considerable heterogeneity in cognitive trajectories; thus, longitudinal modeling of cognitive performance at a group and subject-specific level is required to understand how relevant risk factors affect HIV-infected adults, and compare rates of decline to HIV- populations.

The presented work shows how mixed-effects analysis can be employed to estimate the rate of cognitive decline among ART-adherent HIV+ individuals and HIV- controls, and how known risk factors for cognitive decline in the general population (i.e. dyslipidemia and APOE ε4 genotype) differentially modify the rate of decline among HIV+ individuals over age 50 years. The second part of this thesis describes how we will test the influence of pathological variables and genetic markers (systemic inflammation, CVD risk factors, and APOE ε4 genotype) on cognitive decline, and determine the extent to which these factors account for differences in cognitive trajectories. Modeling longitudinal trajectories of midlife cognitive performance in HIV-infection, and determining the explained versus unexplained variance has important implications as this knowledge can help set HIV-related research priorities related to dementia prevention in old age. Additional value of these studies would be in designing clinical trials for people living with HIV infection. To date, clinical trials for HIV-associated neurocognitive disorders have largely been unsuccessful [74]. A potential reason is due in cognitive heterogeneity; some individuals decline rapidly, others have slower decline, and others remain stable or improve as a result of practice[59]. By identifying the relevant pathological variables, and estimating the contribution of these variables to rate of decline, we may better predict who will likely respond to treatments.

Published guidelines in Alzheimer's disease suggest that the best time to intervene is most likely prior to the onset of clinical symptoms, and this strategy likely will translate to HIV-related cognitive disorders [75]. Thus, early identification of relevant clinical and imaging biomarkers predictive of cognitive decline

in virally suppressed HIV+ populations is required. Given HIV-infected adults older than 55 years comprise one of the fastest growing age groups in the HIV+ population, this thesis highlights the value of longitudinal modeling to uncover determinants contributing to cognitive decline in HIV+ populations [76].

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MACS Study Participants, Coordinators and Administrators

MGH Department of Neuroscience: Merit Cudkowicz, Nagagopal Venna, Tracey Cho

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Elvis Plokhooy, Prosanto Mukerji, Isabella Mukhooy

LIST OF ABBREVIATIONS

AD: Alzheimer's disease

APOE: Apolipoprotein E

ART: antiretroviral therapy

CALCAP: California Computerized Assessment Package

CES-D: Centers for Epidemiologic Studies Depression

CHARTER: CNS HIV Antiretroviral Therapy Effects Research

CNS: central nervous system

CPE: CNS Penetration Effectiveness

ddC: Zalcitabine

ddI: Didanosine

d4T: Stavudine

FAM: 6-carboxyfluorescein

HAND: HIV-1 associated neurocognitive disorders

HCV: hepatitis C virus

HDL: high-density lipoprotein

HIV-1: human immunodeficiency virus-1

IQ: intelligence quotient

IQR: interquartile range

LDL: low-density lipoprotein

MACS: Multicenter AIDS Cohort Study

NNTC: National NeuroAIDS Tissue Consortium

NP: neuropsychological

OI: opportunistic infections

PLWH: people living with HIV infection

RAVLT: Rey Auditory Verbal Learning Test

RNA: ribonucleic acid

SQL: structured query language

SD: standard deviation

SE: standard error

VIC: 4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein

VL: viral load

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